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## GEOCHEMICAL ANALYSIS OF A BEAN SEED IN CERTAIN REGIONS IN THE REPUBLIC OF MACEDONIA

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**A b s t r a c t:** The presence of the macro-elements and elements in traces in the bean seed is determined by ICP-AES (atomic emission spectrometric with inductive plasma). The various bean seeds are taken out of five different types from some regions in the Republic of Macedonia. The results obtained refer to the analyses of 19 elements of the group of macro and micro-elements. There is a wide concentrated range in the samples from 30286,8 mg/kg for K in the test 9 to 0,15 mg/kg for Co in the test 8. More attention is devoted to the presence of Ca, Fe and Zn in the bean seed because it is a valuable source of these macro-elements and elements in traces which are important nutritive items for more than 2 billion people in the world. Therefore, the biological importance of these elements in traces is perceived as well (their transfer and accumulation from the soil into the plants)

**Key words:** bean seed; geochemical analysis

### INTRODUCTION

For more than 300 million people worldwide, beans are cheap food in the daily diet. Ordinary beans (*Phaseolus Vulgaris*) in general are a source of Fe, Zn, Ca and other nutrients that the humans imbibe (Awadallah et al. 1986). The beans imbibition is widespread in Central and South America, East Africa (Sherif et al. 1979) and most countries in Europe and Asia. Beans are a very important product in the nutrition in the Republic of Macedonia. Efforts to improve its nutritional content in terms of protein and minerals, especially Fe, Ca and Zn will be useful in the diet of many people. The analyses of various bio-components of the beans are more focused on the concentration of Fe, Ca and Zn. Much of the research on trace elements show that the average Fe concentration is about 55 mg/kg, but researchers have noted changes in the concentrations of Fe to 100 mg/kg and more.

The average concentration of Zn ranges from 20–60 mg/kg. Also the environment and the genotype influence the concentration of trace elements in the seeds of beans (Shaclette, 1972). Trace elements are extremely important for plant develop-

ment and normal functioning of basic functions. Some trace elements (Fe, Zn, Ca, Mg, P, K and Cu) (Shaclette, 1980) are assumed as necessary for the development of plants in the capacity of food components in the past. In recent times various sensitive analytical methods (ICP-AES) have a different approach towards the determination of trace elements and therefore the significance of many of them is determined with greater confidence and greater success. If the result is well known that the wealth of trace elements significantly depends on the individual genotypes of beans, their generations, and the conditions for development in the environment.

The Enrichment Factor (EF) of 15 elements in the seeds of beans is estimated from the concentration of that element of the sample compared with the concentration of the same element in the soil of the region. The enrichment factor is calculated based on the known ability of transmission and accumulation of elements in the soil and into plant based on the biological activity of plants.

## MATERIALS AND METHODS

The methodology applied to the analyses of the plant samples basically includes:

Samples of plants;

- Preparation of the samples;
- Identification of the presence of macro-elements and trace elements in the plants through the method ICP-AES;
- Interpretation of the obtained results.

### *Samples of plant origin*

Taking the samples can be individual, if taken from one place of the lot or average, if more individual samples (10-25) from different places are taken. Basically it is much better to take an average of samples as in our case because the results are obtained with greater accuracy and provide a greater overview of the overall geochemical interpretation. While testing the material, whether trees, leaves, buds, roots, or, as in our case, the fruits of beans, special methodology is used, and also a special preparation of samples for analytical provision is made. Immediately before the analysis we identified the type and variety of plants that are analyzed. The average is ten samples of beans from several places in the Republic of Macedonia: the villages Karbinci, Tri Češmi, Argulica (Štip), Žiganci (Kočani), Ratavica (Probištip), the Osojnica river (Vinica), Trabatovište (Delčevo); Tetovo; Capari (Resen) and Kavadarci (Bohula village). (Table 1).

Table 1

### *Location of the beans samples collected in the R. Macedonia*

Test	Variety	Location
1.	Tetovec	Karbinci (Štip)
2.	Black-white	Ratavica (Probištip)
3.	Plitkar	Tri Češmi (Štip)
4.	Bounty	Osojnica (Vinica)
5.	Plitkar	Žiganci (Kočani)
6.	Tetovec	Tetovo
7.	Plitkar	Argulica (Štip)
8.	Plitkar	Trabatovište (Delčevo)
9.	Tetovec	Capari (Resen)
10.	Kavadarci	Bohula

### *Preparation of the samples*

For obtaining more accurate results, the preparation of the samples was made with utmost precision and care. The samples for the analysis with ICP-AES method were prepared in three phases as follows:

- We dried the plant specimens in a special dryer.
- In porcelain dish and with grinding machine we pressed the samples in order to obtain a representative sample for laboratory analysis.
- We dissolved the samples in order to be able consequently to determine the elements of the ICP-AES instrument.

### *Drying the plant specimens*

Fresh fruits of vegetables (beans) contain some moisture absorbed from the soil. Therefore, in order to eliminate moisture from the bean samples in the lab we dry them 48 hours in a special dryer at a temperature of 40 °C.

### *Milling and sowing*

Once samples were dry, in the porcelain dishes and with a finely grinding machine we milled them until we got a dust form of samples. (The porcelain dishes are better for pressing the samples because thus the possible contaminations with the mills are avoided.) As a rule, samples need to be ground to sizes of particles of 150 µm and necessarily riddled through a sieve, size of openings smaller than 150 µm. In this way we received a laboratory sub-sample. For further analysis portions of the laboratory sub-sample are taken.

### *Dissolving*

To determine the elements in the tested plant samples that are of interest for us, we dissolved them in a mixture of hydrogen peroxide and nitric acid. For this purpose of the analytical balance we needed to measure 5.0000 g of the lab sub-sample in a cup. Then, we wet the sample gradually with 5 ml HNO<sub>3</sub>, and then slowly stir not to press another 5 ml H<sub>2</sub>O<sub>2</sub>. We covered the glass with watch glass and left it at room temperature until the reaction ceased to be turbulent, then heat edit until

moisture salted. This procedure was repeated twice. After the third addition we added another 5 ml HNO<sub>3</sub> and finally by filtration through the filter paper with a white ribbon resulting solution is gathered in a measure flask of 100 ml. This resulting solution is used for the provision of physical elements that were of interest to us.

#### *ICP-AAS methodology provision*

These are the elements that can be analyzed and identified with the ICP-AES (Si, Al, Fe, Ca, Mg, Na, K, Ti, Mn), and most of the most common trace elements such as Be, Co, Cu, Mn, Ni, Pb, V, Zn. But certainly there are limited storage in terms of elements that can not be determined with satisfying certainty such a halogen elements, and inert gases, O, N, and C. Instruments of the emission analysis of elements with plasma atom often work

at wavelengths of 180–900 nm, because with them non-metals such as S, P, N, C cannot be identified (their most intensive atomic lines lying on the lower wavelengths 180 nm). It is also hard to measure some trace elements that occur in very low concentrations (less than 1 µg/g) in geological samples. ICP-AES technique is not suitable for the provision of heavy alkali metals (Rb and Cs) content in traces, and the analysis of U, Th, W, Ta is usually below the level of detection for ICP-AES. For good analytical capabilities of ICP-AES, an explanation can be found only in the physical properties (temperature, chemical inertness and low emission electromagnetic radiation) of inductive ion plasma obtained by the tangentially introduced argon, which results in the widespread use of the same atomic spectrometry and the specific design of commercial instruments for ICP-AES.

### INTERPRETATION OF THE OBTAINED RESULTS

Macro-elements and trace elements in samples of beans were determined using ICP-AES after the acid digestion. Analytical results for total con-

centration of 19 elements in samples of beans are given in Table 2, where concentrations of each item are determined by the dry weight of the base.

Table 2

*Contents in mg/kg of tested elements in beans*

	1	2	3	4	5	6	7	8	9	10
As	<3,31	<3,31	<3,31	<3,31	<3,31	<3,31	<3,31	<3,31	<3,31	<3,31
Ag	<0,18	<0,18	<0,18	<0,18	<0,18	<0,18	<0,18	<0,18	<0,18	<0,18
Al	1,45	2,38	8,27	7,72	1,38	0,82	0,32	2,33	0,36	1,7
Sr	4,6	5,35	13,59	2,34	4,91	5,23	10,45	4,44	10,37	11,14
Ca	1600,99	1253	2636,85	1126,93	1399,83	2048,89	2367,84	2223,84	2918,85	2411,39
Ba	1,56	0,87	2,51	1,81	0,84	4,76	2,3	2,67	12,02	1,7
Ni	0,72	0,17	2,49	2,92	0,38	0,8	9,16	1,17	4,73	1,83
Mn	15	12,25	18,51	14,96	14,47	19,51	19,44	17,58	33,77	17,48
Fe	62,08	77,7	61,81	96,62	40,91	62,86	44,15	74,05	153,75	69,07
Cr	0,3	0,18	0,34	0,31	0,02	0,11	0,73	0,31	0,28	0,3
Mg	1472,39	1507,7	1557,7	1845,49	1172,51	1749,5	1789,73	1631,94	1856,07	1686,89
Na	27,07	25,39	32,5	25,44	19,92	54,82	50,81	22,26	29,25	68,18
P	4227,04	5730,37	3228,94	5522,39	3702,63	5537,56	4155,8	4470,96	5469,37	5029,23
Zn	44,66	39,98	38,52	39,69	35,51	41,44	69,97	33,95	54,66	51,79
Cu	12,55	12,46	10,91	8,76	4,93	7,59	11,91	9,98	9,79	12,22
Pb	<0,51	<0,51	<0,51	<0,51	0,69	<0,51	<0,51	<0,51	<0,51	<0,51
Cd	<0,18	<0,18	<0,18	<0,18	<0,18	<0,18	<0,18	<0,18	<0,18	<0,18
Co	<0,11	0,19	0,2	0,26	<0,11	<0,11	0,73	0,15	0,21	<0,11
K	24229,2	28441,6	25160,4	29699,7	18060,2	29043,7	25666,5	25972,5	30286,8	29861,7

Allowable levels of representation of nutritional minerals in traces in the seeds of beans are given in Table 3; these values can be used for proper comparison with results obtained for these 10 samples of beans and evaluation of their quality.

Table 3

*Allowed nutritive concentration domains  
in elements of the beans*  
(J. B. Jones, Jr., B. Wolf, and H. A. Mils, 1991)

Elements	Contents
P (%)	0.25–0.75
K (%)	2.2–4.0
Ca (%)	1.5–3.0
Mg (%)	0.25–0.70
Fe (ppm)	50–300
Cu (ppm)	7–30
Zn (ppm)	20–60
Mn (ppm)	50–300

The concentration of various essential minerals in beans, in this research can be outlined as follows:  $K > P > Ca > Mg > Fe > Zn > Mn > Cu$ .

This is similar to the one determined by J. B. Jones, Jr., et al. (1991), only with the difference in the arrangement of Ca-P, Mn-Zn where  $Ca > P$ ,  $Mn > Zn$  applies. It is known that the concentration of metals in the seeds of beans can be changed in accordance with the geographical origin of the beans and the factors associated with the treatment and the relationship of man to plants. In this study, seeds of beans are of similar cultivars, samples were taken in a relatively short period from different regions. Therefore, the statistical characteristics of important mineral content between different samples of beans may be prescribed to different geographic origins. Compared to the average mineral composition of seeds of beans, presented in Table 3, the beans from Macedonia have similar contents of K, P, Fe, Cu and Zn, a significantly reduced content of Mg, Ca, and a slightly reduced content of Mn (Morghan et al., 2002). Heavy and toxic metals such as Cr, Ni, Sr, Co are often associated with high pollution, because the variations of their levels in the seeds of beans from various regions are due to the location of the bean fields and their proximity or distance from roads. We can conclude that the level of heavy and toxic metals in the seeds of the bean varies. Macedonia has excel-

lent conditions for the production of quality fresh food (vegetables and fruit) which has sufficient quality in view of nutritional elements and has a satisfactory low level of toxic elements at any time. Fresh food should be stored in warehouses in cold, dark places until its consumption. Due to the monitoring of the quality of fresh food and monitoring of the presence of mineral nutrients and harmful, hazardous and toxic heavy metals that are contained in it, in many countries around the world analyze these components during the 1, 3, 6, 9 and 12 month in one production year.

#### *Genetic variability in the content of the macro-elements and trace elements*

The first important issue because of which beans can be enriched with the content of the macro-elements and trace elements (Ca, Fe, Zn, etc.) is to determine the degree of association between genetic variability in the type and content of these same macro-elements and trace elements (Anderson et al., 1973). For this purpose, the content of the macro-elements and trace elements in the samples of beans is identified through ICP-AES technique. The representative samples show some variability in the contents of some macro-elements and trace elements in the different genotypes. The analysis of 10 samples of beans, where eight samples are of white grains, showed that the concentration of Zn ranged from 33.95–69.97 ppm with an average value of 46.3125 ppm, while the remaining samples of motley (pegav) bean the concentration of Zn was in the range from 39.69–39.93 ppm with an average of 39.81 ppm. A clear connection of the content of macro-elements and trace elements and the geographical origin was not been established, although plants that originate from several regions of the Republic of Macedonia (Kočani – vill. Žiganci, and vill. Argulica – Štip) show low concentrations of Fe content in comparison with the ones taken from some other regions. Also the analysis of samples of white beans, the concentration of Ca is in the range of 1339.83–2918.85 ppm with an average of 2201.06 ppm, unlike the samples of multicolored beans where the concentration of Ca ranges from 1126.93–1253 ppm with an average of 1189.965 ppm. Therefore we conclude that as in most developing countries thus also in the R. Macedonia there is a reduced content of Ca in the seed of beans, particularly in the genotypes of the darker color of grains. The important issue for increasing the content of macro-elements and trace elements and the extent



of achieved this depends on how stable components are under certain conditions and processes in the environment (Beebe et al., 1999). In some cases the content of macro-elements and trace elements in the seeds of beans can be changed as a result of the effect of different types of soils and geochemical characteristics of the soil. In some experimental trials it was found that the lack of important macro-elements and trace elements in samples of beans is the result of the continuing process of impoverishment of the soil with the same components and the relationship of man to the overall environment. In these calculations, we noticed that a positive coefficient of correlation exists between several important macro-elements and trace elements. Between Fe and Zn the obtained coefficient of correlation is 0.09 between different genotypes, although in many other surveys of beans in the area of the Andes and North America the correlation coefficient is greater than 0,5 (Research on Trace Minerals in Common Bean). The values of the correlation coefficients of macro-elements in our samples of beans are presented in Table 4.

Table 4

*Factor of correlation between the elements in the seeds of beans*

	Mn	Zn	Ca	Mg	K	P
Fe	0.73	0.09	0.30	0.56	0.63	0.58
Mn		0.44	0.77	0.54	0.38	0.18
Zn			0.46	0.51	0.27	0.06
Ca				0.42	0.26	-0.21
Mg					0.85	0.54
K						0.78

The correlation coefficient ( $r$ ) between the individual elements: Mn-Fe ( $r = 0.73$ ), K-Fe ( $r = 0.63$ ), Ca-Mn ( $r = 0.77$ ), K-Mg ( $r = 0.85$ ), PK ( $r = 0.78$ ) is largely presented with high values. The implication of these correlations are certain genetic factors, macro-elements and various trace elements and they are undivided, with increasing content of an element (e.g.: Fe) would result in an increase of content of another element (e.g.: Mn). These high values of correlation coefficient suggest that the physical and chemical factors of the element associations observed in bean seed may be essential for the distribution of elements in soil and plant (the root, stem, leaf and fruit). Some low values of the coefficient of correlation are determined only be-

tween: Zn-Fe ( $r = 0.09$ ), P-Mn ( $r = 0.18$ ), P-Zn ( $r = 0.06$ ).

# *Evaluation of the enrichment factor for the analyzed elements in the beans samples*

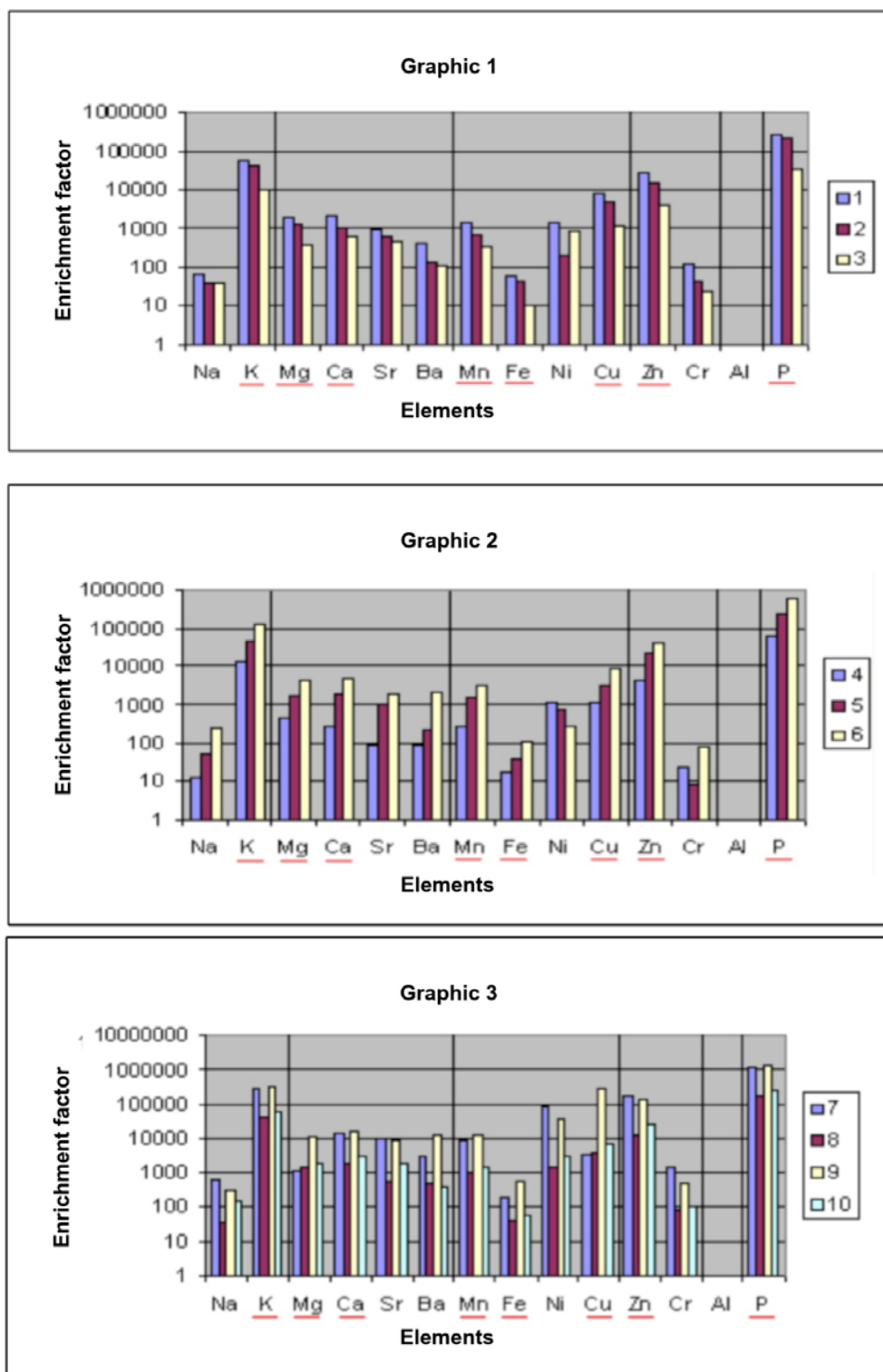
Generally, the plants most inorganic elements are derived from the soil and the elements are absorbed, transferred and accumulated in plants (root, stem, leaf, flower and fruit). Therefore, the concentration of elements in the seeds of beans affects the absorption and accumulative process. The comparison between the enrichment of elements in samples and soil is the basis for the introduction of the enrichment factor ( $EF$  – Enrichment factor) defined with the units frequently used index for geochemical analyses:

$$EF = \frac{[M]_{\text{specimen}} / [Al]_{\text{specimen}}}{[M]_{\text{soil}} / [Al]_{\text{soil}}}$$

where  $[M]$  sample and  $[Al]$  are the concentrations of sample analytical element and aluminium in the sample and  $[M]$  soil and  $[Al]$  soil concentrations of the analytical element and aluminium in the soil. The calculations of the enrichment factor ( $EF$ ), and the concentrations of the analyzed elements are given in Table 2 and used the mean of the elements in ordinary soil (or earth's crust), where the concentration of Al in the sample and the soil (or bark) are used in normal analytical concentrations in them, because Al is one of many inactive elements of the geochemistry. According to the definition of  $EF$ , one can conclude that elements with  $EF > 1$  are more accumulated in seeds of beans compared to the elements included in average soil (Fig. 1, a, b, c). These results suggest that important elements are absorbed from the soil and significantly accumulated in seeds of beans. On the other hand, the values of  $EF$  for Fe, Na and Cr are determined as the lowest compared with the values of  $EF$  for all other elements they suggest that Fe, Na and Cr are accumulated in beans. It should also be the case for the unimportant elements that are present here, such as Sr, Ba, Ni, and Cr, but the obtained values for  $EF$  are larger than one in every ten samples, which indicates active transmission of these elements in beans. This may depend primarily on the specific nature of the bean, although this feature cannot be explained here. Ten different samples of beans that are grown in different soils in different areas are analyzed in the presented analyses because it is difficult to discuss some basic characteristics of these elements to their pri-

marily absorbing, accumulative and biological function. However, the enrichment factor can be used as one of the factors for determining the values of the kinetic behaviors of elements of interest

to us, between plants and soil. Because of all this, the presented methods and the growing development in identical and different soils depend on the multi elementary basis.



**Fig. 1 (a,b,c).** Graphic picture of the enrichment factor

## CONCLUSION

Ten different samples of beans were analyzed by analytical method for multi element determination of the macro-elements and trace elements and also the results are precise and accurate in the digesting and analytical procedures for plant samples. The analytical results for the 19 items specified in the survey are presented in accordance with the values presented in previous research in this field worldwide. Because of all that, the presented analytical method is suitable for the provision of representation of the macro-elements and trace elements in samples of beans using ICP-AES. The content of important minerals (K, P, Mg, Ca, Na,

Fe, Mn, Zn, Cu) in the beans in Macedonia is satisfactory in comparison with results from other countries, excluding the contents of Ca. The level of heavy and toxic metals (Co, Cr, Ni, Ba, Sr) in the seeds of beans in all the samples was satisfactory. The enrichment factor for the various analytical elements in the bean seed is determined in this study. It is concluded that all essential elements for plant growth, except Fe, were accumulated in sufficient copies of all beans. Therefore, the enrichment factor gives some important properties of the schedule for the kinetical behavior of elements in samples of beans absorbed by the soil.

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## Резиме

### ГЕОХЕМИСКИ ИСТРАЖУВАЊА НА СЕМЕ ОД ГРАВ ОД ОДРЕДЕНИ РЕГИОНИ ВО РЕПУБЛИКА МАКЕДОНИЈА

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**Клучни зборови:** семе од грав; геохемиска анализа

Присуството на макро-елементите и елементите во траги во семето од грав е одредено со ICP-AES (атомска емисиона спектрометрија со индуктивна плазма). Земени од пет разни видови семиња грав од неклку региони во Република Македонија. Добиените резултати се однесуваат на анализите на 19 елементи од групата на макро- и микро-елементи. Постои широк опсег на концентрации во примероците, од 30286,8 mg/kg за К во тестот 9 до 0,15

mg/kg за Со во тестот 8. Поголемо внимание е посветено на присуството на Са, Fe и Zn во семето од грав, бидејќи гравот е значаен извор на овие макро-елементи и елементи во траги како важни нутриционистички состојки во исхраната на повеќе од 2 милијарди луѓе во светот. Затоа се земени предвид нивното биолошкото значење, како и трансферот и акумулацијата од почвите во растенијата.